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(71) Applicant *(for all designated States except US)*: MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: GRAHAM, Samuel, L. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).

(74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).

(54) Title: INHIBITORS OF PRENYL–PROTEIN TRANSFERASE

(57) Abstract

The present invention is directed to compounds which inhibit prenyl–protein transferases, farnesyl–protein transferase and geranylgeranyl–protein transferase type I, and the prenylation of the oncogene protein Ras. The invention is further directed to chemotherapeutic compositions containing the compounds of this invention and methods for inhibiting farnesyl–protein transferase and geranylgeranyl–protein transferase type I and the prenylation of the oncogene protein Ras.

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TITLE OF THE INVENTION

INHIBITORS OF PRENYL-PROTEIN TRANSFERASE

BACKGROUND OF THE INVENTION

The present invention relates to certain compounds that are useful for the inhibition of prenyl-protein transferases and the treatment of cancer. In particular, the invention relates to prenyl-protein transferase inhibitors which are efficacious *in vivo* as inhibitors of geranylgeranyl-protein transferase type I (GGTase-I) and that inhibit the cellular processing of both the H-Ras protein and the K4B-Ras protein.

Prenylation of proteins by prenyl-protein transferases represents a class of post-translational modification (Glomset, J. A., Gelb, M. H., and Farnsworth, C. C. (1990). Trends Biochem. Sci. 15, 139-142; Maltese, W. A. (1990). FASEB J. 4, 3319-3328). This modification typically is required for the membrane localization and function of these proteins. Prenylated proteins share characteristic C-terminal sequences including CAAX (C, Cys; A, an aliphatic amino acid; X, another amino acid), XXCC, or XCXC. Three post-translational processing steps have been described for proteins having a C-terminal CAAX sequence: addition of either a 15 carbon (farnesyl) or 20 carbon (geranylgeranyl) isoprenoid to the Cys residue, proteolytic cleavage of the last 3 amino acids, and methylation of the new C-terminal carboxylate (Cox, A. D. and Der, C. J. (1992a). *Critical Rev. Oncogenesis* 3:365-400; Newman, C. M. H. and Magee, A. I. (1993). *Biochim. Biophys. Acta* 1155:79-96). Some proteins may also have a fourth modification: palmitoylation of one or two Cys residues N-terminal to the farnesylated Cys. While some mammalian cell proteins terminating in XCXC are carboxymethylated, it is not clear whether carboxy methylation follows prenylation of proteins terminating with a XXCC motif (Clarke, S. (1992). Annu. Rev. Biochem. 61, 355-386). For all of the prenylated proteins, addition of the isoprenoid is the first step and is required for the subsequent steps (Cox, A. D. and

Der, C. J. (1992a). *Critical Rev. Oncogenesis* 3:365-400; Cox, A. D. and Der, C. J. (1992b) *Current Opinion Cell Biol.* 4:1008-1016).

Three enzymes have been described that catalyze protein prenylation: farnesyl-protein transferase (FPTase), geranylgeranyl-protein transferase type I (GGPTase-I), and geranylgeranyl-protein transferase type-II (GGPTase-II, also called Rab GGPTase). These enzymes are found in both yeast and mammalian cells (Clarke, 1992; Schafer, W. R. and Rine, J. (1992) *Annu. Rev. Genet.* 30:209-237). Each of these enzymes selectively uses farnesyl diphosphate or geranyl-geranyl diphosphate as the isoprenoid donor and selectively recognizes the protein substrate. FPTase farnesylates CaaX-containing proteins that end with Ser, Met, Cys, Gln or Ala. For FPTase, CaaX tetrapeptides comprise the minimum region required for interaction of the protein substrate with the enzyme. The enzymological characterization of these three enzymes has demonstrated that it is possible to selectively inhibit one with little inhibitory effect on the others (Moores, S. L., Schaber, M. D., Mosser, S. D., Rands, E., O'Hara, M. B., Garsky, V. M., Marshall, M. S., Pompliano, D. L., and Gibbs, J. B., *J. Biol. Chem.*, 266:17438 (1991), U.S. Pat. No. 5,470,832).

The prenylation reactions have been shown genetically to be essential for the function of a variety of proteins (Clarke, 1992; Cox and Der, 1992a; Gibbs, J. B. (1991). *Cell* 65: 1-4; Newman and Magee, 1993; Schafer and Rine, 1992). This requirement often is demonstrated by mutating the CaaX Cys acceptors so that the proteins can no longer be prenylated. The resulting proteins are devoid of their central biological activity. These studies provide a genetic "proof of principle" indicating that inhibitors of prenylation can alter the physiological responses regulated by prenylated proteins.

The Ras protein is part of a signaling pathway that links cell surface growth factor receptors to nuclear signals initiating cellular proliferation. Biological and biochemical studies of Ras action indicate that Ras functions like a G-regulatory protein. In the inactive state, Ras is bound to GDP. Upon growth factor receptor activation, Ras is induced to exchange GDP for GTP and undergoes a conformational

change. The GTP-bound form of Ras propagates the growth stimulatory signal until the signal is terminated by the intrinsic GTPase activity of Ras, which returns the protein to its inactive GDP bound form (D.R. Lowy and D.M. Willumsen, *Ann. Rev. Biochem. 62*:851-891 (1993)). Activation of Ras leads to activation of multiple intracellular signal transduction pathways, including the MAP Kinase pathway and the Rho/Rac pathway (Joneson *et al.*, Science *271*:810-812).

Mutated *ras* genes are found in many human cancers, including colorectal carcinoma, exocrine pancreatic carcinoma, and myeloid leukemias. The protein products of these genes are defective in their GTPase activity and constitutively transmit a growth stimulatory signal.

The Ras protein is one of several proteins that are known to undergo post-translational modification. Farnesyl-protein transferase utilizes farnesyl pyrophosphate to covalently modify the Cys thiol group of the Ras CAAX box with a farnesyl group (Reiss *et al., Cell, 62*:81-88 (1990); Schaber *et al., J. Biol. Chem., 265*:14701-14704 (1990); Schafer *et al., Science, 249*:1133-1139 (1990); Manne *et al., Proc. Natl. Acad. Sci USA, 87*:7541-7545 (1990)).

Ras must be localized to the plasma membrane for both normal and oncogenic functions. At least 3 post-translational modifications are involved with Ras membrane localization, and all 3 modifications occur at the C-terminus of Ras. The Ras C-terminus contains a sequence motif termed a "CAAX" or "Cys-Aaa^1-Aaa^2-Xaa" box (Cys is cysteine, Aaa is an aliphatic amino acid, the Xaa is any amino acid) (Willumsen *et al., Nature 310*:583-586 (1984)). Depending on the specific sequence, this motif serves as a signal sequence for the enzymes farnesyl-protein transferase or geranylgeranyl-protein transferase, which catalyze the alkylation of the cysteine residue of the CAAX motif with a C_{15} or C_{20} isoprenoid, respectively. (S. Clarke., *Ann. Rev. Biochem. 61*:355-386 (1992); W.R. Schafer and J. Rine, *Ann. Rev. Genetics 30*:209-237 (1992)). Direct inhibition of farnesyl-protein transferase would be more specific and attended by fewer side effects

than would occur with the required dose of a general inhibitor of isoprene biosynthesis.

Other farnesylated proteins include the Ras-related GTP-binding proteins such as RhoB, fungal mating factors, the nuclear lamins, and the gamma subunit of transducin. James, et al., *J. Biol. Chem. 269*, 14182 (1994) have identified a peroxisome associated protein Pxf which is also farnesylated. James, et al., have also suggested that there are farnesylated proteins of unknown structure and function in addition to those listed above.

Inhibitors of farnesyl-protein transferase (FPTase) have been described in two general classes. The first class includes analogs of farnesyl diphosphate (FPP), while the second is related to protein substrates (e.g., Ras) for the enzyme. The peptide derived inhibitors that have been described are generally cysteine containing molecules that are related to the CAAX motif that is the signal for protein prenylation. (Schaber *et al.*, *ibid*; Reiss *et. al.*, *ibid*; Reiss *et al.*, *PNAS*, *88*:732-736 (1991)). Such inhibitors may inhibit protein prenylation while serving as alternate substrates for the farnesyl-protein transferase enzyme, or may be purely competitive inhibitors (U.S. Patent 5,141,851, University of Texas; N.E. Kohl *et al.*, *Science*, *260*:1934-1937 (1993); Graham, et al., *J. Med. Chem.*, *37*, 725 (1994)).

Mammalian cells express four types of Ras proteins (H-, N-, K4A-, and K4B-Ras) among which K4B-Ras is the most frequently mutated form of Ras in human cancers. The genes that encode these proteins are abbreviated H-*ras*, N-*ras* , K4A-*ras* and K4B-*ras* respectively. H-*ras* is an abbreviation for Harvey-ras. K4A-*ras* and K4B-*ras* are abbreviations for the Kirsten splice variants of *ras* that contain the 4A and 4B exons, respectively. Inhibition of farnesyl-protein transferase has been shown to block the growth of H-*ras*-transformed cells in soft agar and to modify other aspects of their transformed phenotype. It has also been demonstrated that certain inhibitors of farnesyl-protein transferase selectively block the processing of the H-Ras oncoprotein intracellularly (N.E. Kohl *et al.*, *Science*, *260*:1934-1937 (1993) and G.L. James *et al.*, *Science*, *260*:

1937-1942 (1993). Recently, it has been shown that an inhibitor of farnesyl-protein transferase blocks the growth of H-*ras*-dependent tumors in nude mice (N.E. Kohl *et al.*, *Proc. Natl. Acad. Sci U.S.A.*, *91*:9141-9145 (1994) and induces regression of mammary and salivary carcinomas in H-*ras* transgenic mice (N.E. Kohl *et al.*, *Nature Medicine*, 1:792-797 (1995).

Indirect inhibition of farnesyl-protein transferase *in vivo* has been demonstrated with lovastatin (Merck & Co., Rahway, NJ) and compactin (Hancock *et al.*, *ibid*; Casey *et al.*, *ibid*; Schafer *et al.*, *Science 245*:379 (1989)). These drugs inhibit HMG-CoA reductase, the rate limiting enzyme for the production of polyisoprenoids including farnesyl pyrophosphate. Inhibition of farnesyl pyrophosphate biosynthesis by inhibiting HMG-CoA reductase blocks Ras membrane localization in cultured cells.

It has been disclosed that the lysine-rich region and terminal CVIM sequence of the C-terminus of K-RasB confer resistance to inhibition of the cellular processing of that protein by certain selective FPTase inhibitors. James, et al., *J. Biol. Chem. 270*, 6221 (1995) Those FPTase inhibitors were effective in inhibiting the processing of H-Ras proteins. James et al., suggested that prenylation of the K4B-Ras protein by GGTase contributed to the resistance to the selective FPTase inhibitors.

Several groups of scientists have recently disclosed compounds that are non-selective FPTase/GGTase inhibitors. (Nagasu et al. *Cancer Research, 55*:5310-5314 (1995); PCT application WO 95/25086).

It is the object of the instant invention to provide a prenyl-protein transferase inhibitor which is efficacious *in vivo* as an inhibitor of geranylgeranyl-protein transferase type I (GGTase-I), also known as CAAX GGTase.

It is also the object of the present invention to provide a compound which inhibits the cellular processing of both the H-Ras protein and the K4B-Ras protein.

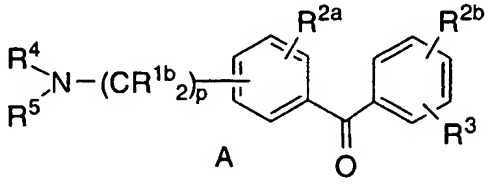
It is also the object of the present invention to provide a compound which is efficacious *in vivo* as an inhibitor of the growth of cancer cells characterized by a mutated K4B-Ras protein.

A composition which comprises such an inhibitor compound is used in the present invention to treat cancer.

SUMMARY OF THE INVENTION

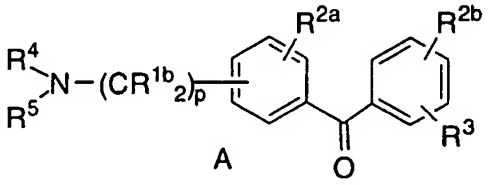
The present invention comprises benzophenone-containing compounds which inhibit the prenyl-protein transferases: farnesyl-protein transferase and geranylgeranyl-protein transferase type I. Further contained in this invention are chemotherapeutic compositions containing these prenyl-protein transferase inhibitors and methods for their production.

The compounds of this invention are illustrated by the formula A:



DETAILED DESCRIPTION OF THE INVENTION

The compounds of this invention are useful in the inhibition of prenyl-protein transferases and the prenylation of the oncogene protein Ras. In a first embodiment of this invention, the inhibitors of prenyl-protein transferases are illustrated by the formula A:



wherein:

R^{1a} is selected from: hydrogen or C_1-C_6 alkyl;

R^{1b} is independently selected from:

a) hydrogen,

b) aryl, heterocycle, cycloalkyl, $R^{10}O$-, -$N(R^{10})_2$ or C_2-C_6 alkenyl,

c) C_1-C_6 alkyl unsubstituted or substituted by aryl, heterocycle, cycloalkyl, alkenyl, $R^{10}O$-, or -$N(R^{10})_2$;

R^{2a}, R^{2b} and R^3 are independently selected from:

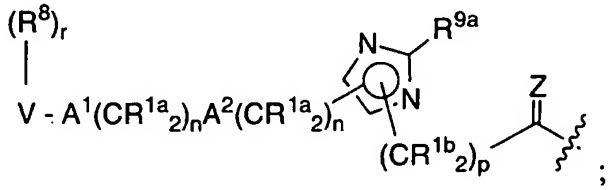
a) hydrogen,

b) C_1-C_6 alkyl unsubstituted or substituted by C_2-C_6 alkenyl, $R^{10}O$-, $R^{11}S(O)_m$-, $R^{10}C(O)NR^{10}$-, CN, N_3, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, $R^{10}OC(O)$-, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-,

c) unsubstituted or substituted aryl, unsubstituted or substituted heterocycle, unsubstituted or substituted cycloalkyl, alkenyl, $R^{10}O$-, $R^{11}S(O)_m$-, $R^{10}C(O)NR^{10}$-, CN, NO_2, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, $R^{10}OC(O)$-, N_3, -$N(R^{10})_2$, halogen or $R^{11}OC(O)NR^{10}$-, and

d) C_1-C_6 alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocyclic and C_3-C_{10} cycloalkyl;

R^4 is



;

R^5 is hydrogen;

R^8 is selected from:

a) hydrogen,
b) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 perfluoroalkyl, F, Cl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, CN, NO_2, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, $R^{10}OC(O)$-, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-, and
c) C_1-C_6 alkyl substituted by C_1-C_6 perfluoroalkyl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, $R^{10}OC(O)$-, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-;

R^{9a} is independently selected from C_1-C_6 alkyl and aryl;

R^{10} is independently selected from hydrogen, C_1-C_6 alkyl, C_1-C_6 perfluoroalkyl, 2,2,2-trifluoroethyl, benzyl and aryl;

R^{11} is independently selected from C_1-C_6 alkyl, benzyl and aryl;

A^1 and A^2 are independently selected from: a bond, -CH=CH-, -C≡C-, -C(O)-, $-C(O)NR^8$-, $-NR^8C(O)$-, O, $-N(R^8)$-, $-S(O)_2N(R^8)$-, $-N(R^8)S(O)_2$-, or $S(O)_m$;

V is selected from:
a) hydrogen,
b) heterocycle selected from pyrrolidinyl, imidazolyl, pyridinyl, thiazolyl, pyridonyl, 2-oxopiperidinyl, indolyl, quinolinyl, isoquinolinyl, and thienyl,
c) aryl,
d) C_1-C_{20} alkyl wherein from 0 to 4 carbon atoms are replaced with a heteroatom selected from O, S, and N, and
e) C_2-C_{20} alkenyl,

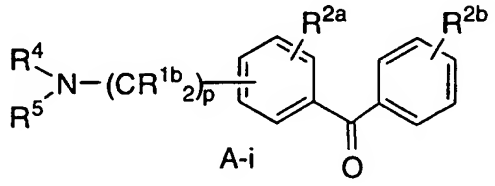
provided that V is not hydrogen if A^1 is $S(O)_m$ and V is not hydrogen if A^1 is a bond, n is 0 and A^2 is $S(O)_m$;

Z is H_2 or O;

m is 0, 1 or 2;
n is 0, 1, 2, 3 or 4;
p is independently 0, 1, 2, 3 or 4; and
r is 0 to 5, provided that r is 0 when V is hydrogen;

or the pharmaceutically acceptable salts thereof.

A preferred embodiment of the compounds of this invention is illustrated by the following formula A-i:



wherein:

R^{1b} is independently selected from:

a) hydrogen,
b) aryl, heterocycle, cycloalkyl, $R^{10}O$-, -$N(R^{10})_2$ or C_2-C_6 alkenyl,
c) C_1-C_6 alkyl unsubstituted or substituted by aryl, heterocycle, cycloalkyl, alkenyl, $R^{10}O$-, or -$N(R^{10})_2$;

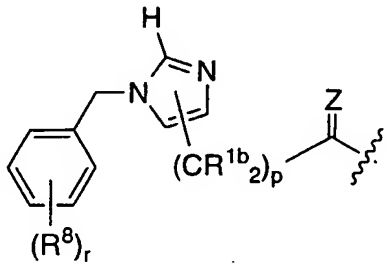
R^{2a} and R^{2b} are independently selected from:

a) hydrogen,
b) C_1-C_6 alkyl unsubstituted or substituted by C_2-C_6 alkenyl, $R^{10}O$-, $R^{11}S(O)_m$-, $R^{10}C(O)NR^{10}$-, CN, N_3, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, $R^{10}OC(O)$-, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-,
c) unsubstituted or substituted aryl, unsubstituted or substituted heterocycle, unsubstituted or substituted cycloalkyl, alkenyl, $R^{10}O$-, $R^{11}S(O)_m$-, $R^{10}C(O)NR^{10}$-, CN, NO_2,

$(R^{10})_2N$-C(NR^{10})-, $R^{10}C(O)$-, $R^{10}OC(O)$-, N_3, -$N(R^{10})_2$, halogen or $R^{11}OC(O)NR^{10}$-, and

d) C_1-C_6 alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocyclic and C_3-C_{10} cycloalkyl;

R^4 is



;

R^5 is hydrogen;

R^8 is independently selected from:

a) hydrogen,

b) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 perfluoroalkyl, F, Cl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, CN, NO_2, $(R^{10})_2N$-C(NR^{10})-, $R^{10}C(O)$-, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-, and

c) C_1-C_6 alkyl substituted by C_1-C_6 perfluoroalkyl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, $(R^{10})_2N$-C(NR^{10})-, $R^{10}C(O)$-, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-;

R^{10} is independently selected from hydrogen, C_1-C_6 alkyl, substituted or unsubstituted C_1-C_6 aralkyl and substituted or unsubstituted aryl;

R^{11} is independently selected from C_1-C_6 alkyl, benzyl and aryl;

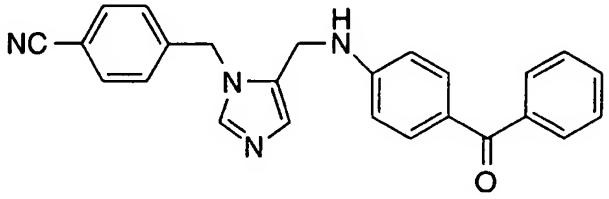
Z is H_2 or O;

m is 0, 1 or 2;
n is 0, 1, 2, 3 or 4;
p is independently 0, 1 or 2; and
r is 0 to 5;

or the pharmaceutically acceptable salts thereof.

The preferred compound of this invention is as follows:

4-[((1-(4-cyanobenzyl)-5-imidazolyl)methyl)amino]benzophenone



or the pharmaceutically acceptable salts or optical isomers thereof.

The compounds of the instant invention differ from previously disclosed benzophenone-containing compounds, (U.S. Pat. No. 5,710,171 - January 20, 1998; PCT Publ. No. WO 97/36886 - October 9, 1997; PCT Publ. No. WO 97/36875 - October 9, 1997; PCT Publ. No. WO 97/36585 - October 9, 1997) that were described as selective inhibitors of farnesyl-protein transferase, in that the instant compounds are dual inhibitors of farnesyl-protein transferase and geranylgeranyl-protein transferase type I (GGTase-I). Preferably, the compounds of the instant invention inhibit FPTase *in vitro* (Example 2) at an IC_{50} of less than 1 μM, inhibit GGTase-I *in vitro* (Example 3) at an IC_{50} of less than 1 μM and inhibited the cellular processing (farnesylation) of H-Ras (Example 4) at an IC_{50} of less than 1 μM.

The compounds of the present invention may have asymmetric centers and occur as racemates, racemic mixtures, and as individual diastereomers, with all possible isomers, including optical isomers, being included in the present invention. When any variable (e.g. aryl, heterocycle, R^1, R^2 etc.) occurs more than one time in any constituent, its definition on each occurrence is independent at every

other occurrence. Also, combinations of substituents/or variables are permissible only if such combinations result in stable compounds.

As used herein, "alkyl" is intended to include both branched and straight-chain saturated aliphatic hydrocarbon groups having the specified number of carbon atoms; "alkoxy" represents an alkyl group of indicated number of carbon atoms attached through an oxygen bridge. "Halogen" or "halo" as used herein means fluoro, chloro, bromo and iodo.

As used herein, "aryl" is intended to mean any stable monocyclic or bicyclic carbon ring of up to 7 members in each ring, wherein at least one ring is aromatic. Examples of such aryl elements include phenyl, naphthyl, tetrahydronaphthyl, indanyl, biphenyl, phenanthryl, anthryl or acenaphthyl.

The term heterocycle or heterocyclic, as used herein, represents a stable 5- to 7-membered monocyclic or stable 8- to 11-membered bicyclic heterocyclic ring which is either saturated or unsaturated, and which consists of carbon atoms and from one to four heteroatoms selected from the group consisting of N, O, and S, and including any bicyclic group in which any of the above-defined heterocyclic rings is fused to a benzene ring. The heterocyclic ring may be attached at any heteroatom or carbon atom which results in the creation of a stable structure. Examples of such heterocyclic elements include, but are not limited to, azepinyl, benzimidazolyl, benzisoxazolyl, benzofurazanyl, benzopyranyl, benzothiopyranyl, benzofuryl, benzothiazolyl, benzothienyl, benzoxazolyl, chromanyl, cinnolinyl, dihydrobenzofuryl, dihydrobenzothienyl, dihydrobenzothiopyranyl, dihydrobenzothiopyranyl sulfone, furyl, imidazolidinyl, imidazolinyl, imidazolyl, indolinyl, indolyl, isochromanyl, isoindolinyl, isoquinolinyl, isothiazolidinyl, isothiazolyl, isothiazolidinyl, morpholinyl, naphthyridinyl, oxadiazolyl, 2-oxoazepinyl, oxazolyl, 2-oxopiperazinyl, 2-oxopiperdinyl, 2-oxopyrrolidinyl, piperidyl, piperazinyl, pyridyl, pyrazinyl, pyrazolidinyl, pyrazolyl, pyridazinyl, pyrimidinyl, pyrrolidinyl, pyrrolyl, quinazolinyl, quinolinyl, quinoxalinyl, tetrahydrofuryl, tetrahydroisoquinolinyl, tetrahydroquinolinyl,

thiamorpholinyl, thiamorpholinyl sulfoxide, thiazolyl, thiazolinyl, thienofuryl, thienothienyl, and thienyl.

As used herein, "heteroaryl" is intended to mean any stable monocyclic or bicyclic carbon ring of up to 7 members in each ring, wherein at least one ring is aromatic and wherein from one to four carbon atoms are replaced by heteroatoms selected from the group consisting of N, O, and S. Examples of such heterocyclic elements include, but are not limited to, benzimidazolyl, benzisoxazolyl, benzofurazanyl, benzopyranyl, benzothiopyranyl, benzofuryl, benzothiazolyl, benzothienyl, benzoxazolyl, chromanyl, cinnolinyl, dihydrobenzofuryl, dihydrobenzothienyl, dihydrobenzothiopyranyl, dihydrobenzothiopyranyl sulfone, furyl, imidazolyl, indolinyl, indolyl, isochromanyl, isoindolinyl, isoquinolinyl, isothiazolyl, naphthyridinyl, oxadiazolyl, pyridyl, pyrazinyl, pyrazolyl, pyridazinyl, pyrimidinyl, pyrrolyl, quinazolinyl, quinolinyl, quinoxalinyl, tetrahydroisoquinolinyl, tetrahydroquinolinyl, thiazolyl, thienofuryl, thienothienyl, and thienyl.

As used herein in the definition of R^2 and R^4, the term "the substituted group" intended to mean a substituted C_{1-8} alkyl, substituted C_{2-8} alkenyl, substituted C_{2-8} alkynyl, substituted aryl or substituted heterocycle from which the substituent(s) R^2 and R^3 are selected.

As used herein in the definition of R^6, R^{6a}, R^7 and R^{7a}, the substituted C_{1-8} alkyl, substituted C_{3-6} cycloalkyl, substituted aroyl, substituted aryl, substituted heteroaroyl, substituted arylsulfonyl, substituted heteroarylsulfonyl and substituted heterocycle include moieties containing from 1 to 3 substituents in addition to the point of attachment to the rest of the compound. Preferably, such substituents are selected from the group which includes but is not limited to F, Cl, Br, CF_3, NH_2, $N(C_1\text{-}C_6$ alkyl$)_2$, NO_2, CN, (C_1-C_6 alkyl)O-, -OH, (C_1-C_6 alkyl)S(O)$_m$-, (C_1-C_6 alkyl)C(O)NH-, H_2N-C(NH)-, (C_1-C_6 alkyl)C(O)-, (C_1-C_6 alkyl)OC(O)-, N_3,(C_1-C_6 alkyl)OC(O)NH-, phenyl, pyridyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, thienyl, furyl, isothiazolyl and C_1-C_{20} alkyl.

Lines drawn into the ring systems from substituents (such as from R^{2a}, R^{2b}, R^3 etc.) indicate that the indicated bond may be attached to any of the substitutable ring carbon atoms.

Preferably, R^{1a} is hydrogen.

Preferably, R^{1b} is independently selected from: hydrogen, $-N(R^{10})_2$, $R^{10}O-$ or unsubstituted or substituted C_1-C_6 alkyl wherein the substituent on the substituted C_1-C_6 alkyl is selected from phenyl, $-N(R^{10})_2$ and $R^{10}O-$.

Preferably, R^{2a} and R^{2b} are independently selected from: H, unsubstituted or substituted C_1-C_6 alkyl, $R^{10}O-$ and halogen.

Preferably, R^3 is selected from: hydrogen and C_1-C_6 alkyl.

Preferably, R^{9a} is hydrogen or methyl. Most preferably, R^{9a} is hydrogen.

Preferably, R^{10} is selected from H, C_1-C_6 alkyl and benzyl.

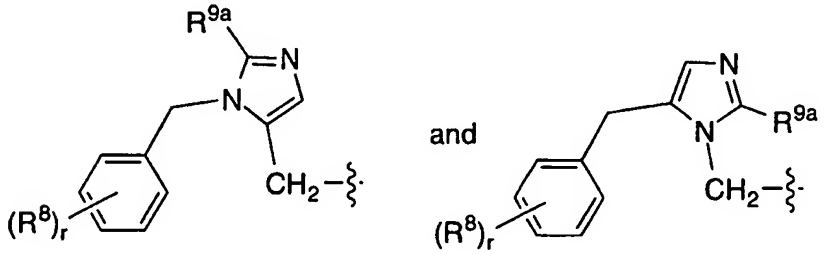
Preferably, A^1 and A^2 are independently selected from: a bond, $-C(O)NR^{10}-$, $-NR^{10}C(O)-$, O, $-N(R^{10})-$, $-S(O)_2N(R^{10})-$ and $-N(R^{10})S(O)_2-$. Most preferably, A^1 and A^2 are a bond.

Preferably, V is selected from hydrogen, heterocycle and aryl. More preferably, V is phenyl.

Preferably, n and r are independently 0, 1, or 2.

Preferably p is 1, 2 or 3.

Preferably, R^4 is selected from:



It is intended that the definition of any substituent or variable (e.g., R^{1a}, R^9, n, etc.) at a particular location in a molecule be independent of its definitions elsewhere in that molecule. Thus,

-$N(R^{10})_2$ represents -NHH, $-NHCH_3$, $-NHC_2H_5$, etc. It is understood that substituents and substitution patterns on the compounds of the instant invention can be selected by one of ordinary skill in the art to provide compounds that are chemically stable and that can be readily synthesized by techniques known in the art, as well as those methods set forth below, from readily available starting materials.

The pharmaceutically acceptable salts of the compounds of this invention include the conventional non-toxic salts of the compounds of this invention as formed, e.g., from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like: and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxy-benzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, trifluoroacetic and the like.

The pharmaceutically acceptable salts of the compounds of this invention can be synthesized from the compounds of this invention which contain a basic moiety by conventional chemical methods. Generally, the salts are prepared either by ion exchange chromatography or by reacting the free base with stoichiometric amounts or with an excess of the desired salt-forming inorganic or organic acid in a suitable solvent or various combinations of solvents.

Reactions used to generate the compounds of this invention are prepared by employing reactions as shown in the Schemes 1-15, in addition to other standard manipulations such as ester hydrolysis, cleavage of protecting groups, etc., as may be known in the literature or exemplified in the experimental procedures. Substituent R, as shown in the Schemes, represents the substituents R^2, R^3, R^4, and R^5; however the point of attachment to the ring is illustrative only and is not meant to be limiting.

These reactions may be employed in a linear sequence

to provide the compounds of the invention or they may be used to synthesize fragments which are subsequently joined by the reductive alkylation reactions described in the Schemes.

Synopsis of Schemes 1-3:

The requisite intermediates are in some cases commercially available, or can be prepared according to literature procedures, for the most part.

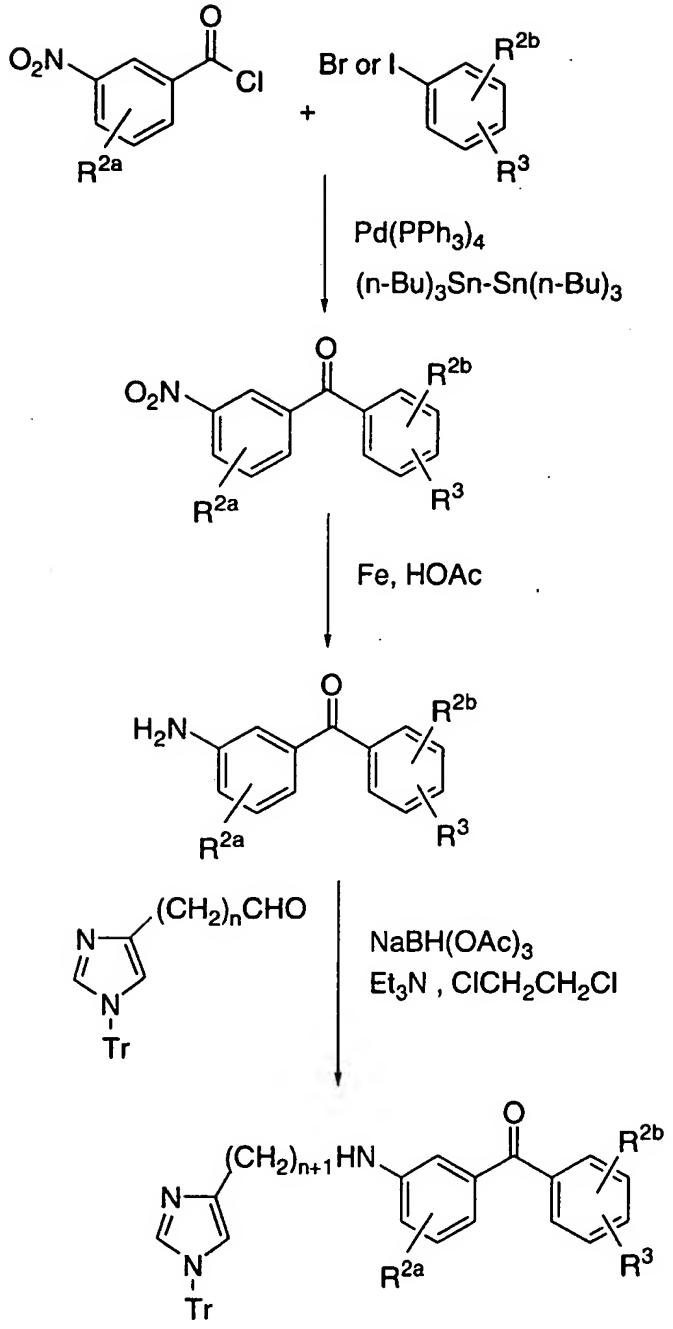
The prenyl transferase inhibitors of formula (A) can be synthesized in accordance with Schemes 1-3 below, in addition to other standard manipulations such as ester hydrolysis, cleavage of protecting groups, etc., as may be known in the literature or exemplified in the experimental procedures. Some key reactions utilized to form the aminodiphenyl moiety of the instant compounds are shown.

The reactions may be employed in a linear sequence to provide the compounds of the invention or they may be used to synthesize fragments which are subsequently joined by the alkylation reactions described in the Reaction Scheme.

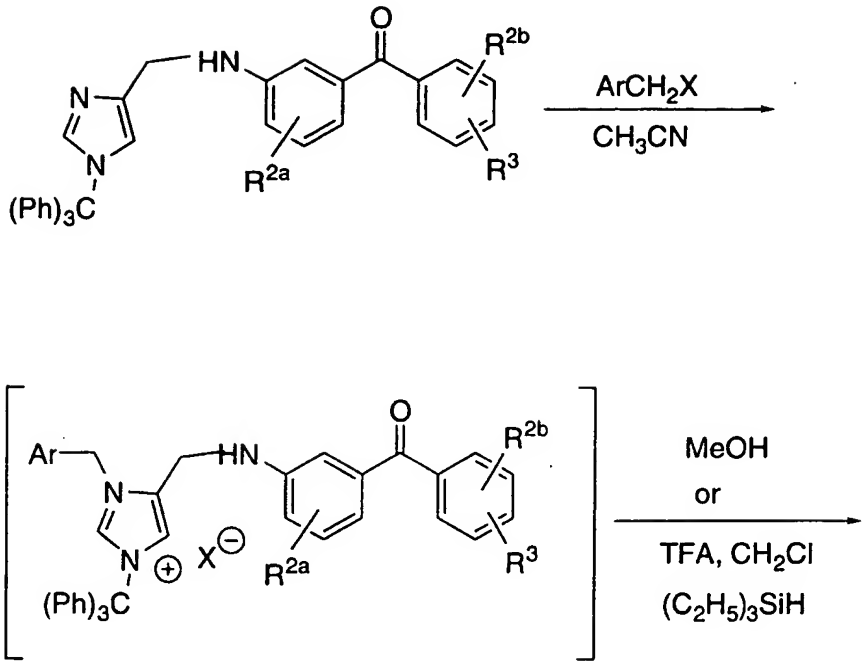
A method of forming the benzophenone intermediates, illustrated in Scheme 1, is a Stille reaction with an aryl stannane. Such amine intermediates may then be reacted as illustrated hereinabove with a variety of aldehydes and esters/acids.

Imidazole acetic acid can be converted to the acetate by standard procedures, and the acetate can be first reacted with an alkyl halide, then treated with refluxing methanol to provide the regiospecifically alkylated imidazole acetic acid ester (Scheme 3). Hydrolysis and reaction with benzophenone amine in the presence of condensing reagents such as 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) leads to acylated product.

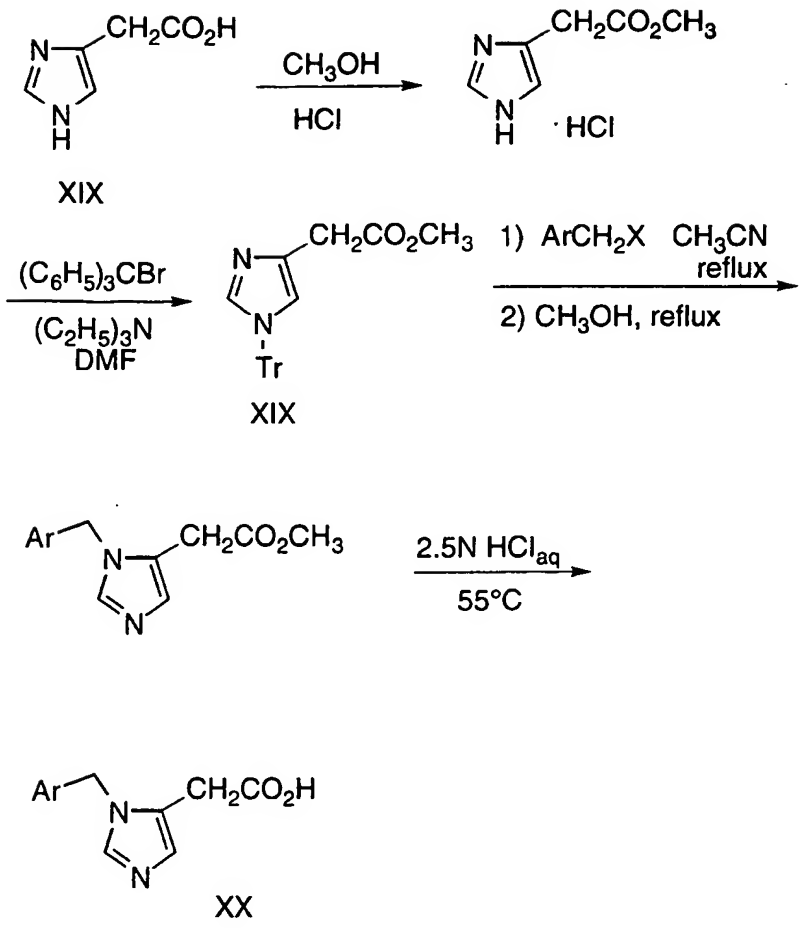
SCHEME 1


O
O_2N
Cl
R^{2a}
+
Br or I
R^{2b}
R^3
$Pd(PPh_3)_4$
$(n\text{-}Bu)_3Sn\text{-}Sn(n\text{-}Bu)_3$
H_2N
Fe, HOAc
$(CH_2)_nCHO$
N
N
Tr
$NaBH(OAc)_3$
Et_3N , $ClCH_2CH_2Cl$
$(CH_2)_{n+1}HN$

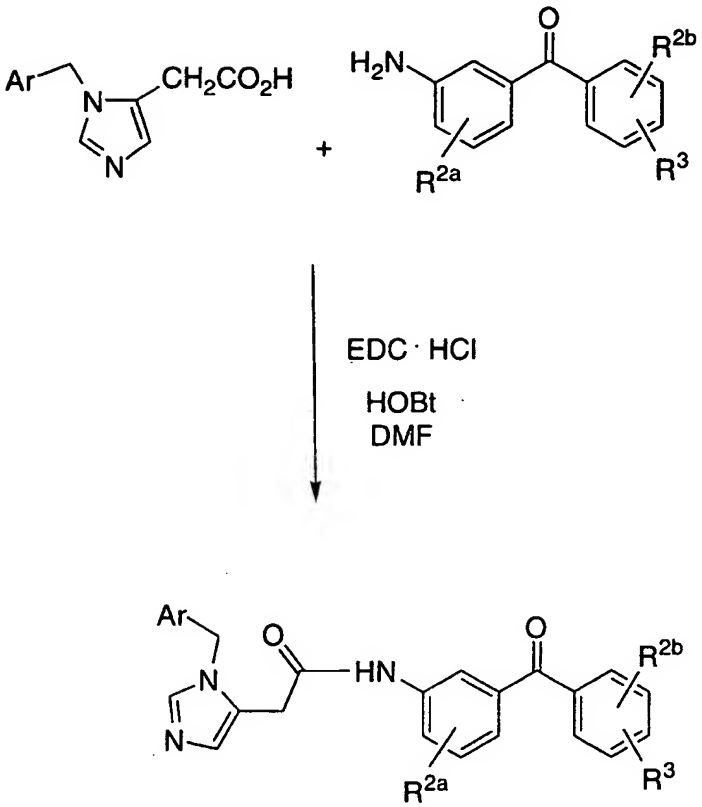
SCHEME 2


HN
O
R2b
R2a
R3
N
N
(Ph)3C
ArCH2X
CH3CN
Ar
X⊖
MeOH
or
TFA, CH2Cl
(C2H5)3SiH

SCHEME 3


CH2CO2H
N
N
H
CH3OH
HCl
CH2CO2CH3
N
N
H
·HCl
XIX
(C6H5)3CBr
(C2H5)3N
DMF
CH2CO2CH3
N
N
Tr
XIX
1) ArCH2X CH3CN
reflux
2) CH3OH, reflux
Ar
N
N
CH2CO2CH3
2.5N HClaq
55°C
Ar
N
N
CH2CO2H
XX

SCHEME 3 (continued)



The instant compounds are useful as pharmaceutical agents for mammals, especially for humans. These compounds may be administered to patients for use in the treatment of cancer. Examples of the type of cancer which may be treated with the compounds of this invention include, but are not limited to, colorectal carcinoma, exocrine pancreatic carcinoma, myeloid leukemias and neurological tumors. Such tumors may arise by mutations in the *ras* genes themselves, mutations in the proteins that can regulate Ras activity (i.e., neurofibromin (NF-1), neu, src, abl, lck, fyn) or by other mechanisms.

The compounds of the instant invention inhibit prenyl-protein transferase and the prenylation of the oncogene protein Ras. The instant compounds may also inhibit tumor angiogenesis, thereby affecting the growth of tumors (J. Rak et al. *Cancer Research*, 55:

4575-4580 (1995)). Such anti-angiogenesis properties of the instant compounds may also be useful in the treatment of certain forms of vision deficit related to retinal vascularization.

The compounds of this invention are also useful for inhibiting other proliferative diseases, both benign and malignant, wherein Ras proteins are aberrantly activated as a result of oncogenic mutation in other genes (i.e., the Ras gene itself is not activated by mutation to an oncogenic form) with said inhibition being accomplished by the administration of an effective amount of the compounds of the invention to a mammal in need of such treatment. For example, a component of NF-1 is a benign proliferative disorder.

The instant compounds may also be useful in the treatment of certain viral infections, in particular in the treatment of hepatitis delta and related viruses (J.S. Glenn et al. *Science*, 256:1331-1333 (1992).

The compounds of the instant invention are also useful in the prevention of restenosis after percutaneous transluminal coronary angioplasty by inhibiting neointimal formation (C. Indolfi et al. *Nature medicine*, 1:541-545(1995).

The instant compounds may also be useful in the treatment and prevention of polycystic kidney disease (D.L. Schaffner et al. *American Journal of Pathology*, 142:1051-1060 (1993) and B. Cowley, Jr. et al.*FASEB Journal*, 2:A3160 (1988)).

The instant compounds may also be useful for the treatment of fungal infections.

The instant compounds may also be useful as inhibitors of proliferation of vascular smooth muscle cells and therefore useful in the prevention and therapy of arteriosclerosis and diabetic vascular pathologies.

The compounds of this invention may be administered to mammals, preferably humans, either alone or, preferably, in combination with pharmaceutically acceptable carriers, excipients or diluents, in a pharmaceutical composition, according to standard pharmaceutical practice. The compounds can be administered orally or

parenterally, including the intravenous, intramuscular, intraperitoneal, subcutaneous, rectal and topical routes of administration.

The pharmaceutical compositions containing the active ingredient may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, microcrystalline cellulose, sodium crosscarmellose, corn starch, or alginic acid; binding agents, for example starch, gelatin, polyvinyl-pyrrolidone or acacia, and lubricating agents, for example, magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to mask the unpleasant taste of the drug or delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a water soluble taste masking material such as hydroxypropylmethylcellulose or hydroxypropylcellulose, or a time delay material such as ethyl cellulose, cellulose acetate buryrate may be employed.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water soluble carrier such as polyethyleneglycol or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

Aqueous suspensions contain the active material in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethyl-cellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally-occurring phosphatide, for example lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethylene-oxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose, saccharin or aspartame.

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as butylated hydroxyanisol or alpha-tocopherol.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present. These compositions

may be preserved by the addition of an anti-oxidant such as ascorbic-- acid.

The pharmaceutical compositions of the invention may also be in the form of an oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally-occurring phosphatides, for example soy bean lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening, flavouring agents, preservatives and antioxidants.

Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, flavoring and coloring agents and antioxidant.

The pharmaceutical compositions may be in the form of a sterile injectable aqueous solutions. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution.

The sterile injectable preparation may also be a sterile injectable oil-in-water microemulsion where the active ingredient is dissolved in the oily phase. For example, the active ingredient may be first dissolved in a mixture of soybean oil and lecithin. The oil solution then introduced into a water and glycerol mixture and processed to form a microemulation.

The injectable solutions or microemulsions may be introduced into a patient's blood-stream by local bolus injection. Alternatively, it may be advantageous to administer the solution or microemulsion in such a way as to maintain a constant circulating concentration of the instant compound. In order to maintain such a constant concentration, a continuous intravenous delivery device may be utilized. An example of such a device is the Deltec CADD-PLUS™ model 5400 intravenous pump.

The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleagenous suspension for intramuscular and subcutaneous administration. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butane diol. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Compounds of Formula A may also be administered in the form of a suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include cocoa butter, glycerinated gelatin, hydrogenated vegetable oils, mixtures of polyethylene glycols of various molecular weights and fatty acid esters of polyethylene glycol.

For topical use, creams, ointments, jellies, solutions or suspensions, etc., containing the compound of Formula A are employed. (For purposes of this application, topical application shall include mouth washes and gargles.)

The compounds for the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles and delivery devices, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in the art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen.

As used herein, the term "composition" is intended to encompass a product comprising the specified ingredients in the specific

amounts, as well as any product which results, directly or indirectly, from combination of the specific ingredients in the specified amounts.

When a compound according to this invention is administered into a human subject, the daily dosage will normally be determined by the prescribing physician with the dosage generally varying according to the age, weight, sex and response of the individual patient, as well as the severity of the patient's symptoms.

In one exemplary application, a suitable amount of compound is administered to a mammal undergoing treatment for cancer. Administration occurs in an amount between about 0.1 mg/kg of body weight to about 60 mg/kg of body weight per day, preferably of between 0.5 mg/kg of body weight to about 40 mg/kg of body weight per day.

The compounds of the instant invention may also be co-administered with other well known therapeutic agents that are selected for their particular usefulness against the condition that is being treated. For example, the compounds of the instant invention may also be co-administered with other well known cancer therapeutic agents that are selected for their particular usefulness against the condition that is being treated. Included in such combinations of therapeutic agents are combinations of the instant farnesyl-protein transferase inhibitors and an antineoplastic agent. It is also understood that such a combination of antineoplastic agent and inhibitor of farnesyl-protein transferase may be used in conjunction with other methods of treating cancer and/or tumors, including radiation therapy and surgery.

Examples of an antineoplastic agent include, in general, microtubule-stabilizing agents (such as paclitaxel (also known as Taxol®), docetaxel (also known as Taxotere®), epothilone A, epothilone B, desoxyepothilone A, desoxyepothilone B or their derivatives); microtubule-disruptor agents; alkylating agents, anti-metabolites; epidophyllotoxin; an antineoplastic enzyme; a topoisomerase inhibitor; procarbazine; mitoxantrone; platinum coordination complexes; biological response modifiers and growth

inhibitors; hormonal/anti-hormonal therapeutic agents and haematopoietic growth factors.

Example classes of antineoplastic agents include, for example, the anthracycline family of drugs, the vinca drugs, the mitomycins, the bleomycins, the cytotoxic nucleosides, the taxanes, the epothilones, discodermolide, the pteridine family of drugs, diynenes and the podophyllotoxins. Particularly useful members of those classes include, for example, doxorubicin, carminomycin, daunorubicin, aminopterin, methotrexate, methopterin, dichloro-methotrexate, mitomycin C, porfiromycin, 5-fluorouracil, 6-mercaptopurine, gemcitabine, cytosine arabinoside, podophyllotoxin or podo-phyllotoxin derivatives such as etoposide, etoposide phosphate or teniposide, melphalan, vinblastine, vincristine, leurosidine, vindesine, leurosine, paclitaxel and the like. Other useful antineoplastic agents include estramustine, cisplatin, carboplatin, cyclophosphamide, bleomycin, tamoxifen, ifosamide, melphalan, hexamethyl melamine, thiotepa, cytarabin, idatrexate, trimetrexate, dacarbazine, L-asparaginase, camptothecin, CPT-11, topotecan, ara-C, bicalutamide, flutamide, leuprolide, pyridobenzoindole derivatives, interferons and interleukins.

The preferred class of antineoplastic agents is the taxanes and the preferred antineoplastic agent is paclitaxel.

Radiation therapy, including x-rays or gamma rays which are delivered from either an externally applied beam or by implantation of tiny radioactive sources, may also be used in combination with the instant inhibitor of farnesyl-protein transferase alone to treat cancer.

Additionally, compounds of the instant invention may also be useful as radiation sensitizers, as described in WO 97/38697, published on October 23, 1997, and herein incorporated by reference.

The instant compounds may also be useful in combination with other inhibitors of parts of the signaling pathway that links cell surface growth factor receptors to nuclear signals initiating cellular proliferation. Thus, the instant compounds may be utilized in combination with farnesyl pyrophosphate competitive inhibitors of the activity of farnesyl-protein transferase or in combination with a

compound which has Raf antagonist activity. The instant compounds may also be co-administered with compounds that are selective inhibitors of geranylgeranyl protein transferase or farnesyl-protein transferase.

In particular, the compounds disclosed in the following patents and publications may be useful as farnesyl pyrophosphate-competitive inhibitor component of the instant composition: U.S. Ser. Nos. 08/254,228 and 08/435,047. Those patents and publications are incorporated herein by reference.

In practicing methods of this invention, which comprise administering, simultaneously or sequentially or in any order, two or more of a protein substrate-competitive inhibitor and a farnesyl pyrophosphate-competitive inhibitor, such administration can be orally or parenterally, including intravenous, intramuscular, intraperitoneal, subcutaneous, rectal and topical routes of administration. It is preferred that such administration be orally. It is more preferred that such administration be orally and simultaneously. When the protein substrate-competitive inhibitor and farnesyl pyrophosphate-competitive inhibitor are administered sequentially, the administration of each can be by the same method or by different methods.

The instant compounds may also be useful in combination with an integrin antagonist for the treatment of cancer, as described in U.S. Ser. No. 09/055,487, filed April 6, 1998, which is incorporated herein by reference.

As used herein the term an integrin antagonist refers to compounds which selectively antagonize, inhibit or counteract binding of a physiological ligand to an integrin(s) that is involved in the regulation of angiogenisis, or in the growth and invasiveness of tumor cells. In particular, the term refers to compounds which selectively antagonize, inhibit or counteract binding of a physiological ligand to the $\alpha v\beta 3$ integrin, which selectively antagonize, inhibit or counteract binding of a physiological ligand to the $\alpha v\beta 5$ integrin, which antagonize, inhibit or counteract binding of a physiological ligand to both the $\alpha v\beta 3$ integrin and the $\alpha v\beta 5$ integrin, or which antagonize,

inhibit or counteract the activity of the particular integrin(s) expressed on capillary endothelial cells. The term also refers to antagonists of the $\alpha1\beta1$, $\alpha2\beta1$, $\alpha5\beta1$, $\alpha6\beta1$ and $\alpha6\beta4$ integrins. The term also refers to antagonists of any combination of $\alpha v\beta3$ integrin, $\alpha v\beta5$ integrin, $\alpha1\beta1$, $\alpha2\beta1$, $\alpha5\beta1$, $\alpha6\beta1$ and $\alpha6\beta4$ integrins. The instant compounds may also be useful with other agents that inhibit angiogenisis and thereby inhibit the growth and invasiveness of tumor cells, including, but not limited to angiostatin and endostatin.

Similarly, the instant compounds may be useful in combination with agents that are effective in the treatment and prevention of NF-1, restenosis, polycystic kidney disease, infections of hepatitis delta and related viruses and fungal infections.

If formulated as a fixed dose, such combination products employ the combinations of this invention within the dosage range described below and the other pharmaceutically active agent(s) within its approved dosage range. Combinations of the instant invention may alternatively be used sequentially with known pharmaceutically acceptable agent(s) when a multiple combination formulation is inappropriate.

EXAMPLES

Examples provided are intended to assist in a further understanding of the invention. Particular materials employed, species and conditions are intended to be further illustrative of the invention and not limitative of the reasonable scope thereof.

EXAMPLES 1

4-[((1-(4-cyanobenzyl)-5-imidazolyl)methyl)amino]benzophenone hydrochloride

Step A: Preparation of 1-triphenylmethyl-4-(hydroxymethyl)-imidazole

To a solution of 4-(hydroxymethyl)imidazole hydrochloride (35.0 g, 260 mmol) in 250 mL of dry DMF at room temperature was added triethylamine (90.6 mL, 650 mmol). A white solid precipitated from the solution. Chlorotriphenylmethane (76.1 g, 273 mmol) in 500 mL of DMF was added dropwise. The reaction mixture was stirred for 20 hours, poured over ice, filtered, and washed with ice water. The resulting product was slurried with cold dioxane, filtered, and dried *in vacuo* to provide the titled product as a white solid which was sufficiently pure for use in the next step.

Step B: Preparation of 1-triphenylmethyl-4-(acetoxymethyl)-imidazole

Alcohol from Step A (260 mmol, prepared above) was suspended in 500 mL of pyridine. Acetic anhydride (74 mL, 780 mmol) was added dropwise, and the reaction was stirred for 48 hours during which it became homogeneous. The solution was poured into 2 L of EtOAc, washed with water (3 x 1 L), 5% aq. HCl soln. (2 x 1 L), sat. aq. $NaHCO_3$, and brine, then dried (Na_2SO_4), filtered, and concentrated *in vacuo* to provide the crude product. The acetate was isolated as a white powder which was sufficiently pure for use in the next reaction.

Step C: Preparation of 1-(4-cyanobenzyl)-5-(acetoxymethyl)-imidazole hydrobromide

A solution of the product from Step B (85.8 g, 225 mmol) and α-bromo-*p*-tolunitrile (50.1 g, 232 mmol) in 500 mL of EtOAc was stirred at 60°C for 20 hours, during which a pale yellow precipitate formed. The reaction was cooled to room temperature and filtered to provide the solid imidazolium bromide salt. The filtrate was concentrated *in vacuo* to a volume 200 mL, reheated at 60°C for two hours, cooled to room temperature, and filtered again. The filtrate was concentrated *in vacuo* to a volume 100 mL, reheated at 60°C for another two hours, cooled to room temperature, and concentrated *in vacuo* to provide a pale yellow solid. All of the solid

material was combined, dissolved in 500 mL of methanol, and warmed to 60°C. After two hours, the solution was reconcentrated *in vacuo* to provide a white solid which was triturated with hexane to remove soluble materials. Removal of residual solvents *in vacuo* provided the titled product hydrobromide as a white solid which was used in the next step without further purification.

Step D: Preparation of 1-(4-cyanobenzyl)-5-(hydroxymethyl)-imidazole

To a solution of the acetate from Step C (50.4 g, 150 mmol) in 1.5 L of 3:1 THF/water at 0°C was added lithium hydroxide monohydrate (18.9 g, 450 mmol). After one hour, the reaction was concentrated *in vacuo*, diluted with EtOAc (3 L), and washed with water, sat. aq. $NaHCO_3$ and brine. The solution was then dried (Na_2SO_4), filtered, and concentrated *in vacuo* to provide the crude product as a pale yellow fluffy solid which was sufficiently pure for use in the next step without further purification.

Step E: Preparation of 1-(4-cyanobenzyl)-5-imidazolecarboxaldehyde

To a solution of the alcohol from Step D (21.5 g, 101 mmol) in 500 mL of DMSO at room temperature was added triethylamine (56 mL, 402 mmol), then SO_3-pyridine complex (40.5 g, 254 mmol). After 45 minutes, the reaction was poured into 2.5 L of EtOAc, washed with water (4 x 1 L) and brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to provide the aldehyde as a white powder which was sufficiently pure for use in the next step without further purification.

Step F: Preparation of 4-[((1-(4-cyanobenzyl)-5-imidazolyl)methyl)amino]benzophenone hydrochloride

The titled product was prepared by reductive alkylation of the 1-(4-cyanobenzyl)-5-imidazolecarboxaldehyde prepared as described in Step E (124 mg, 0.588 mmol) and 4-aminobenzophenone (116 mg, 0.588 mmol) using the following procedure: To a solution

of the amine in 200 mL of 1,2-dichloroethane at 0°C was added 4Å powdered molecular sieves (200 mg), followed by sodium triacetoxyborohydride (0.186 g, 0.882 mmol). The imidazole carboxaldehyde from Step E was added, and the reaction was stirred at 0°C. After 26 hours, the reaction was poured into EtOAc, washed with dilute aq. $NaHCO_3$, and the aqueous layer was back-extracted with EtOAc. The combined organics were washed with brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification by flash column chromatography through silica gel (2-6% $MeOH/CH_2Cl_2$) and conversion to the hydrochloride salt provided the titled product as a white solid (126 mg, 50% yield). FAB ms (m+1) 393.11.

Anal. Calc. for $C_{25}H_{20}N_5O \cdot 1.40HCl \cdot 0.40H_2O$:

C, 66.62; H, 4.96; N, 12.43.

Found: C, 66.73; H, 4.94; N, 12.46.

EXAMPLE 2

In vitro inhibition of ras farnesyl transferase

Transferase Assays. Isoprenyl-protein transferase activity assays are carried out at 30 °C unless noted otherwise. A typical reaction contains (in a final volume of 50 µL): [^{3}H]farnesyl diphosphate, Ras protein , 50 mM HEPES, pH 7.5, 5 mM $MgCl_2$, 5 mM dithiothreitol, 10 µM $ZnCl_2$, 0.1% polyethyleneglycol (PEG) (15,000-20,000 mw) and isoprenyl-protein transferase. The FPTase employed in the assay is prepared by recombinant expression as described in Omer, C.A., Kral, A.M., Diehl, R.E., Prendergast, G.C., Powers, S., Allen, C.M., Gibbs, J.B. and Kohl, N.E. (1993) *Biochemistry* **32**:5167-5176. After thermally pre-equilibrating the assay mixture in the absence of enzyme, reactions are initiated by the addition of isoprenyl-protein transferase and stopped at timed intervals (typically 15 min) by the addition of 1 M HCl in ethanol (1 mL). The quenched reactions are allowed to stand for 15 m (to complete the precipitation process). After adding 2 mL of 100% ethanol, the reactions are vacuum-filtered through Whatman GF/C filters. Filters are washed

four times with 2 mL aliquots of 100% ethanol, mixed with scintillation fluid (10 mL) and then counted in a Beckman LS3801 scintillation counter.

For inhibition studies, assays are run as described above, except inhibitors are prepared as concentrated solutions in 100% dimethyl sulfoxide and then diluted 20-fold into the enzyme assay mixture. IC_{50} values are determined with both transferase substrates near K_M concentrations. Substrate conditions for inhibitor IC_{50} determinations are as follows: FTase, 650 nM Ras-CVLS (SEQ.ID.NO.: 1), 100 nM farnesyl diphosphate.

The compound of the instant invention described in the above Example 1 was tested for inhibitory activity against human FPTase by the assay described above and was found to have IC_{50} of 50 μM.

EXAMPLE 3

Modified*In vitro* GGTase inhibition assay

The modified geranylgeranyl-protein transferase inhibition assay is carried out at room temperature. A typical reaction contains (in a final volume of 50 μL): [^{3}H]geranylgeranyl diphosphate, biotinylated Ras peptide, 50 mM HEPES, pH 7.5, a modulating anion (for example 10 mM glycerophosphate or 5mM ATP), 5 mM $MgCl_2$, 10 μM $ZnCl_2$, 0.1% PEG (15,000-20,000 mw), 2 mM dithiothreitol, and geranylgeranyl-protein transferase type I(GGTase). The GGTase-type I enzyme employed in the assay is prepared as described in U.S. Pat. No. 5,470,832, incorporated by reference. The Ras peptide is derived from the K4B-Ras protein and has the following sequence: biotinyl-GKKKKKKSKTKCVIM (single amino acid code) (SEQ.ID.NO.: 2). Reactions are initiated by the addition of GGTase and stopped at timed intervals (typically 15 min) by the addition of 200 μL of a 3 mg/mL suspension of streptavidin SPA beads (Scintillation Proximity Assay beads, Amersham) in 0.2 M sodium phosphate, pH 4, containing 50 mM EDTA, and 0.5% BSA. The quenched reactions are

allowed to stand for 2 hours before analysis on a Packard TopCount -- scintillation counter.

For inhibition studies, assays are run as described above, except inhibitors are prepared as concentrated solutions in 100% dimethyl sulfoxide and then diluted 25-fold into the enzyme assay mixture. IC_{50} values are determined with Ras peptide near K_M concentrations. Enzyme and substrate concentrations for inhibitor IC_{50} determinations are as follows: 75 pM GGTase-I, 1.6 μM Ras peptide, 100 nM geranylgeranyl diphosphate.

EXAMPLE 4

Cell-based*in vitro* ras farnesylation assay

The cell line used in this assay is a v-ras line derived from either Rat1 or NIH3T3 cells, which expressed viral Ha-ras p21. The assay is performed essentially as described in DeClue, J.E. et al., Cancer Research 51:712-717, (1991). Cells in 10 cm dishes at 50-75% confluency are treated with the test compound (final concentration of solvent, methanol or dimethyl sulfoxide, is 0.1%). After 4 hours at 37°C, the cells are labeled in 3 ml methionine-free DMEM supplemeted with 10% regular DMEM, 2% fetal bovine serum and 400 mCi[^{35}S]methionine (1000 Ci/mmol). After an additional 20 hours, the cells are lysed in 1 ml lysis buffer (1% NP40/20 mM HEPES, pH 7.5/5 mM $MgCl_2$/1mM DTT/10 mg/ml aprotinen/2 mg/ml leupeptin/2 mg/ml antipain/0.5 mM PMSF) and the lysates cleared by centrifugation at 100,000 x g for 45 min. Aliquots of lysates containing equal numbers of acid-precipitable counts are bought to 1 ml with IP buffer (lysis buffer lacking DTT) and immunoprecipitated with the ras-specific monoclonal antibody Y13-259 (Furth, M.E. et al., J. Virol. 43:294-304, (1982)). Following a 2 hour antibody incubation at 4°C, 200 ml of a 25% suspension of protein A-Sepharose coated with rabbit anti rat IgG is added for 45 min. The immunoprecipitates are washed four times with IP buffer (20 nM HEPES, pH 7.5/1 mM EDTA/1% Triton X-100.0.5% deoxycholate/0.1%/SDS/0.1 M NaCl) boiled in SDS-PAGE

sample buffer and loaded on 13% acrylamide gels. When the dye front reached the bottom, the gel is fixed, soaked in Enlightening, dried and autoradiographed. The intensities of the bands corresponding to farnesylated and nonfarnesylated ras proteins are compared to determine the percent inhibition of farnesyl transfer to protein.

EXAMPLE 5

Cell-based*in vitro* growth inhibition assay

To determine the biological consequences of FPTase inhibition, the effect of the compounds of the instant invention on the anchorage-independent growth of Rat1 cells transformed with either a v-*ras*, v-*raf*, or v-*mos* oncogene is tested. Cells transformed by v-Raf and v-Mos maybe included in the analysis to evaluate the specificity of instant compounds for Ras-induced cell transformation.

Rat 1 cells transformed with either v-ras, v-raf, or v-mos are seeded at a density of 1 x 10^4 cells per plate (35 mm in diameter) in a 0.3% top agarose layer in medium A (Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum) over a bottom agarose layer (0.6%). Both layers contain 0.1% methanol or an appropriate concentration of the instant compound (dissolved in methanol at 1000 times the final concentration used in the assay). The cells are fed twice weekly with 0.5 ml of medium A containing 0.1% methanol or the concentration of the instant compound. Photomicrographs are taken 16 days after the cultures are seeded and comparisons are made.

EXAMPLE 6

Construction of SEAP reporter plasmid pDSE100

The SEAP reporter plasmid, pDSE100 was constructed by ligating a restriction fragment containing the SEAP coding sequence into the plasmid pCMV-RE-AKI. The SEAP gene is derived from the plasmid pSEAP2-Basic (Clontech, Palo Alto, CA). The plasmid pCMV-RE-AKI was constructed by Deborah Jones (Merck) and contains

5 sequential copies of the 'dyad symmetry response element' cloned upstream of a 'CAT-TATA' sequence derived from the cytomegalovirus immediate early promoter. The plasmid also contains a bovine growth hormone poly-A sequence.

The plasmid, pDSE100 was constructed as follows. A restriction fragment encoding the SEAP coding sequence was cut out of the plasmid pSEAP2-Basic using the restriction enzymes EcoR1 and HpaI. The ends of the linear DNA fragments were filled in with the Klenow fragment of E. coli DNA Polymerase I. The 'blunt ended' DNA containing the SEAP gene was isolated by electrophoresing the digest in an agarose gel and cutting out the 1694 base pair fragment. The vector plasmid pCMV-RE-AKI was linearized with the restriction enzyme Bgl-II and the ends filled in with Klenow DNA Polymerase I. The SEAP DNA fragment was blunt end ligated into the pCMV-RE-AKI vector and the ligation products were transformed into DH5-alpha E. coli cells (Gibco-BRL). Transformants were screened for the proper insert and then mapped for restriction fragment orientation. Properly oriented recombinant constructs were sequenced across the cloning junctions to verify the correct sequence. The resulting plasmid contains the SEAP coding sequence downstream of the DSE and CAT-TATA promoter elements and upstream of the BGH poly-A sequence.

Cloning of a Myristylated viral-H-*ras* expression plasmid

A DNA fragment containing viral-H-*ras* can be PCRed from plasmid "H-1" (Ellis R. et al. J. Virol. **36**, 408, 1980) or "HB-11 (deposited in the ATCC under Budapest Treaty on August 27, 1997, and designated ATCC 209,218) using the following oligos.

Sense strand:

5'TCTCCTCGAGGCCACCATGGGGAGTAGCAAGAGCAAGCCTAA GGACCCCAGCCAGCGCCGGATGACAGAATACAAGCTTGTGGTG G 3'. (SEQ.ID.NO.: 3)

Antisense:

5'CACATCTAGATCAGGACAGCACAGACTTGCAGC 3'. (SEQ.ID.NO.: 4)

A sequence encoding the first 15 aminoacids of the v-src gene, containing a myristylation site, is incorporated into the sense strand oligo. The sense strand oligo also optimizes the 'Kozak' translation initiation sequence immediately 5' to the ATG start site. To prevent prenylation at the viral-*ras* C-terminus, cysteine 186 would be mutated to a serine by substituting a G residue for a C residue in the C-terminal antisense oligo. The PCR primer oligos introduce an XhoI site at the 5' end and a XbaI site at the 3'end. The XhoI-XbaI fragment can be ligated into the mammalian expression plasmid pCI (Promega) cut with XhoI and XbaI. This results in a plasmid in which the recombinant myr-viral-H-ras gene is constitutively transcribed from the CMV promoter of the pCI vector.

Cloning of a viral-H-*ras*-CVLL expression plasmid

A viral-H-*ras* clone with a C-terminal sequence encoding the amino acids CVLL can be cloned from the plasmid "H-1" (Ellis R. et al. J. Virol. **36**, 408, 1980) by PCR using the following oligos.

Sense strand:
5'TCTCCTCGAGGCCACCATGACAGAATACAAGCTTGTGGTGG-3' (SEQ.ID.NO.: 5)

Antisense strand:
5'CACTCTAGACTGGTGTCAGAGCAGCACACACTTGCAGC-3' (SEQ.ID.NO.: 6)

The sense strand oligo optimizes the 'Kozak' sequence and adds an XhoI site. The antisense strand mutates serine 189 to leucine and adds an XbaI site. The PCR fragment can be trimmed with XhoI and XbaI and ligated into the XhoI-XbaI cut vector pCI (Promega). This results in a plasmid in which the mutated viral-H-*ras*-CVLL gene is constitutively transcribed from the CMV promoter of the pCI vector.

Cloning of c-H-*ras*-Leu61 expression plasmid

The human c-H-*ras* gene can be PCRed from a human cerebral cortex cDNA library (Clontech) using the following oligonucleotide primers.

Sense strand:
5'-GAGAGAATTCGCCACCATGACGGAATATAAGCTGGTGG-3'
(SEQ.ID.NO.: 7)

Antisense strand:
5'-GAGAGTCGACGCGTCAGGAGAGCACACACTTGC-3'
(SEQ.ID.NO.: 8)

The primers will amplify a c-H-*ras* encoding DNA fragment with the primers contributing an optimized 'Kozak' translation start sequence, an EcoRI site at the N-terminus and a Sal I stite at the C-terminal end. After trimming the ends of the PCR product with EcoRI and Sal I, the c-H-ras fragment can be ligated into an EcoRI -Sal I cut mutagenesis vector pAlter-1 (Promega). Mutation of glutamine-61 to a leucine can be accomplished using the manufacturer's protocols and the following oligonucleotide:

5'-CCGCCGGCCTGGAGGAGTACAG-3' (SEQ.ID.NO.: 11)

After selection and sequencing for the correct nucleotide substitution, the mutated c-H-*ras*-Leu61 can be excised from the pAlter-1 vector, using EcoRI and Sal I, and be directly ligated into the vector pCI (Promega) which has been digested with EcoRI and Sal I. The new recombinant plasmid will constitutively transcribe c-H-*ras*-Leu61 from the CMV promoter of the pCI vector.

Cloning of a c-N-*ras*-Val-12 expression plasmid

The human c-N-*ras* gene can be PCRed from a human cerebral cortex cDNA library (Clontech) using the following oligonucleotide primers.

Sense strand:
5'-GAGAGAATTCGCCACCATGACTGAGTACAAACTGGTGG-3'
(SEQ.ID.NO.: 9)

Antisense strand:
5'-GAGAGTCGACTTGTTACATCACCACACATGGC-3'
(SEQ.ID.NO.: 10)

The primers will amplify a c-N-*ras* encoding DNA fragment with the primers contributing an optimized 'Kozak' translation start sequence, an EcoRI site at the N-terminus and a Sal I stite at the C-terminal end. After trimming the ends of the PCR product with EcoRI and Sal I, the c-N-ras fragment can be ligated into an EcoRI -Sal I cut mutagenesis vector pAlter-1 (Promega). Mutation of glycine-12 to a valine can be accomplished using the manufacturer's protocols and the following oligonucleotide:

5'-GTTGGAGCAGTTGGTGTTGGG-3' (SEQ.ID.NO.: 11)

After selection and sequencing for the correct nucleotide substitution, the mutated c-N-*ras*-Val-12 can be excised from the pAlter-1 vector, using EcoRI and Sal I, and be directly ligated into the vector pCI (Promega) which has been digested with EcoRI and Sal I. The new recombinant plasmid will constitutively transcribe c-N-*ras*-Val-12 from the CMV promoter of the pCI vector.

Cloning of a c-K-*ras*-Val-12 expression plasmid

The human c-K-*ras* gene can be PCRed from a human cerebral cortex cDNA library (Clontech) using the following oligonucleotide primers.

Sense strand:
5'-GAGAGGTACCGCCACCATGACTGAATATAAACTTGTGG-3'
(SEQ.ID.NO.: 12)

Antisense strand:
5'-CTCTGTCGACGTATTTACATAATTACACACTTTGTC-3'
(SEQ.ID.NO.: 13)

The primers will amplify a c-K-*ras* encoding DNA fragment with the primers contributing an optimized 'Kozak' translation start sequence, a KpnI site at the N-terminus and a Sal I stite at the C-terminal end. After trimming the ends of the PCR product with Kpn I and Sal I, the c-K-*ras* fragment can be ligated into a KpnI -Sal I cut mutagenesis vector pAlter-1 (Promega). Mutation of cysteine-12 to a valine can be accomplished using the manufacturer's protocols and the following oligonucleotide:

5'-GTAGTTGGAGCTGTTGGCGTAGGC-3' (SEQ.ID.NO.: 14)

After selection and sequencing for the correct nucleotide substitution, the mutated c-K-*ras*-Val-12 can be excised from the pAlter-1 vector, using KpnI and Sal I, and be directly ligated into the vector pCI (Promega) which has been digested with KpnI and Sal I. The new recombinant plasmid will constitutively transcribe c-K-*ras*-Val-12 from the CMV promoter of the pCI vector.

SEAP assay

Human C33A cells (human epitheial carcenoma - ATTC collection) are seeded in 10cm tissue culture plates in DMEM + 10% fetal calf serum + 1X Pen/Strep + 1X glutamine + 1X NEAA. Cells are grown at 37°C in a 5% CO_2 atmosphere until they reach 50 -80% of confluency.

The transient transfection is performed by the $CaPO_4$ method (Sambrook et al., 1989). Thus, expression plasmids for H-*ras*, N-*ras*, K-*ras*, Myr-*ras* or H-*ras*-CVLL are co-precipitated with the DSE-SEAP reporter construct. For 10cm plates 600µl of $CaCl_2$-DNA solution is added dropwise while vortexing to 600µl of 2X HBS buffer to give 1.2ml of precipitate solution (see recipes below). This is allowed to sit at room temperature for 20 to 30 minutes. While the precipitate is forming, the media on the C33A cells is replaced with DMEM (minus phenol red; Gibco cat. # 31053-028)+ 0.5% charcoal stripped calf serum + 1X (Pen/Strep, Glutamine and nonessential

aminoacids). The $CaPO_4$-DNA precipitate is added dropwise to the cells and the plate rocked gently to distribute. DNA uptake is allowed to proceed for 5-6 hrs at 37°C under a 5% CO_2 atmosphere.

Following the DNA incubation period, the cells are washed with PBS and trypsinized with 1ml of 0.05% trypsin. The 1 ml of trypsinized cells is diluted into 10ml of phenol red free DMEM + 0.2% charcoal stripped calf serum + 1X (Pen/Strep, Glutamine and NEAA). Transfected cells are plated in a 96 well microtiter plate (100µl/well) to which drug, diluted in media, has already been added in a volume of 100µl. The final volume per well is 200µl with each drug concentration repeated in triplicate over a range of half-log steps.

Incubation of cells and drugs is for 36 hrs at 37° under CO_2. At the end of the incubation period, cells are examined microscopically for evidence of cell distress. Next, 100µl of media containing the secreted alkaline phosphatase is removed from each well and transferred to a microtube array for heat treatment at 65°C for 1 hr to inactivate endogenous alkaline phosphatases (but not the heat stable secreted phosphatase).

The heat treated media is assayed for alkaline phosphatase by a luminescence assay using the luminescence reagent CSPD® (Tropix, Bedford, Mass.). A volume of 50 µl media is combinRased with 200 µl of CSPD cocktail and incubated for 60 minutes at room temperature. Luminesence is monitored using an ML2200 microplate luminometer (Dynatech). Luminescence reflects the level of activation of the fos reporter construct stimulated by the transiently expressed protein.

<u>DNA-$CaPO_4$ precipitate for 10cm. plate of cells</u>

Ras expression plasmid (1µg/µl)	10µl
DSE-SEAP Plasmid (1µg/µl)	2µl
Sheared Calf Thymus DNA (1µg/µl)	8µl
2M $CaCl_2$	74µl
dH_2O	506µl

2X HBS Buffer

280mM NaCl
10mM KCl
1.5mM Na_2HPO_4 $2H_2O$
12mM dextrose
50mM HEPES
Final pH = 7.05

Luminesence Buffer (26ml)

Assay Buffer	20ml
Emerald Reagent™ (Tropix)	2.5ml
100mM homoarginine	2.5ml
CSPD Reagent® (Tropix)	1.0ml

Assay Buffer

Add 0.05M Na_2CO_3 to 0.05M $NaHCO_3$ to obtain pH 9.5.
Make 1mM in $MgCl_2$

EXAMPLE 7

The processing assays employed are modifications of that described by DeClue et al [Cancer Research 51, 712-717, 1991].

K4B-Ras processing inhibition assay

PSN-1 (human pancreatic carcinoma) or viral-K4B-*ras*-transformed Rat1 cells are used for analysis of protein processing. Subconfluent cells in 100 mm dishes are fed with 3.5 ml of media (methionine-free RPMI supplemented with 2% fetal bovine serumor cysteine-free/methionine-free DMEM supplemented with 0.035 ml of 200 mM glutamine (Gibco), 2% fetal bovine serum, respectively) containing the desired concentration of test compound, lovastatin or solvent alone. Cells treated with lovastatin (5-10 μM), a compound that blocks Ras processing in cells by inhibiting a rate-limiting step in the isoprenoid biosynthetic pathway, serve as a positive control. Test compounds are prepared as 1000x concentrated solutions in DMSO to

yield a final solvent concentration of 0.1%. Following incubation at 37°C for two hours 204 μCi/ml [^{35}S]Pro-Mix (Amersham, cell labeling grade) is added.

After introducing the label amino acid mixture, the cells are incubated at 37°C for an additional period of time (typically 6 to 24 hours). The media is then removed and the cells are washed once with cold PBS. The cells are scraped into 1 ml of cold PBS, collected by centrifugation (10,000 x g for 10 sec at room temperature), and lysed by vortexing in 1 ml of lysis buffer (1% Nonidet P-40, 20 mM HEPES, pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.5% deoxycholate, 0.1% SDS, 1 mM DTT, 10 μg/ml AEBSF, 10 μg/ml aprotinin, 2 μg/ml leupeptin and 2 μg/ml antipain). The lysate is then centrifuged at 15,000 x g for 10 min at 4°C and the supernatant saved.

For immunoprecipitation of Ki4B-Ras, samples of lysate supernatant containing equal amounts of protein are utilized. Protein concentration is determined by the bradford method utilizing bovine serum albumin as a standard. The appropriate volume of lysate is brought to 1 ml with lysis buffer lacking DTT and 8 μg of the pan Ras monoclonal antibody, Y13-259, added. The protein/antibody mixture is incubated on ice at 4°C for 24 hours. The immune complex is collected on pansorbin (Calbiochem) coated with rabbit antiserum to rat IgG (Cappel) by tumbling at 4°C for 45 minutes. The pellet is washed 3 times with 1 ml of lysis buffer lacking DTT and protease inhibitors and resuspended in 100 μl elution buffer (10 mM Tris pH 7.4, 1% SDS). The Ras is eluted from the beads by heating at 95°C for 5 minutes, after which the beads are pelleted by brief centrifugation (15,000 x g for 30 sec. at room temperature).

The supernatant is added to 1 ml of Dilution Buffer 0.1% Triton X-100, 5 mM EDTA, 50 mM NaCl, 10 mM Tris pH 7.4) with 2 μg Kirsten-ras specific monoclonal antibody, c-K-ras Ab-1 (Calbiochem). The second protein/antibody mixture is incubated on ice at 4°C for 1-2 hours. The immune complex is collected on pansorbin (Calbiochem) coated with rabbit antiserum to rat IgG (Cappel) by tumbling at 4°C for 45 minutes. The pellet is washed 3 times with 1 ml

of lysis buffer lacking DTT and protease inhibitors and resuspended in Laemmli sample buffer. The Ras is eluted from the beads by heating at 95°C for 5 minutes, after which the beads are pelleted by brief centrifugation. The supernatant is subjected to SDS-PAGE on a 12% acrylamide gel (bis-acrylamide:acrylamide, 1:100), and the Ras visualized by fluorography.

EXAMPLE 8

Rap1 processing inhibition assay

Protocol A:

Cells are labeled, incubated and lysed as described in Example 7.

For immunoprecipitation of Rap1, samples of lysate supernatant containing equal amounts of protein are utilized. Protein concentration is determined by the bradford method utilizing bovine serum albumin as a standard. The appropriate volume of lysate is brought to 1 ml with lysis buffer lacking DTT and 2 µg of the Rap1 antibody, Rap1/Krev1 (121) (Santa Cruz Biotech), is added. The protein/antibody mixture is incubated on ice at 4°C for 1 hour. The immune complex is collected on pansorbin (Calbiochem) by tumbling at 4°C for 45 minutes. The pellet is washed 3 times with 1 ml of lysis buffer lacking DTT and protease inhibitors and resuspended in 100 µl elution buffer (10 mM Tris pH 7.4, 1% SDS). The Rap1 is eluted from the beads by heating at 95°C for 5 minutes, after which the beads are pelleted by brief centrifugation (15,000 x g for 30 sec. at room temperature).

The supernatant is added to 1 ml of Dilution Buffer (0.1% Triton X-100, 5 mM EDTA, 50 mM NaCl, 10 mM Tris pH 7.4) with 2 µg Rap1 antibody, Rap1/Krev1 (121) (Santa Cruz Biotech). The second protein/antibody mixture is incubated on ice at 4°C for 1-2 hours. The immune complex is collected on pansorbin (Calbiochem) by tumbling at 4°C for 45 minutes. The pellet is washed 3 times with 1 ml of lysis buffer lacking DTT and protease inhibitors and resuspended in Laemmli sample buffer. The Rap1 is eluted from the beads by heating at 95°C for

5 minutes, after which the beads are pelleted by brief centrifugation. The supernatant is subjected to SDS-PAGE on a 12% acrylamide gel (bis-acrylamide:acrylamide, 1:100), and the Rap1 visualized by fluorography.

Protocol B:

PSN-1 cells are passaged every 3-4 days in 10cm plates, splitting near-confluent plates 1:20 and 1:40. The day before the assay is set up, 5x 10^6 cells are plated on 15cm plates to ensure the same stage of confluency in each assay. The media for these cells is RPM1 1640 (Gibco), with 15% fetal bovine serum and 1x Pen/Strep antibiotic mix.

The day of the assay, cells are collected from the 15cm plates by trypsinization and diluted to 400,000 cells/ml in media. 0.5ml of these diluted cells are added to each well of 24-well plates, for a final cell number of 200,000 per well. The cells are then grown at 37°C overnight.

The compounds to be assayed are diluted in DMSO in 1/2-log dilutions. The range of final concentrations to be assayed is generally 0.1-100μM. Four concentrations per compound is typical. The compounds are diluted so that each concentration is 1000x of the final concentration (i.e., for a 10μM data point, a 10mM stock of the compound is needed).

2μL of each 1000x compound stock is diluted into 1ml media to produce a 2X stock of compound. A vehicle control solution (2μL DMSO to 1ml media), is utilized. 0.5 ml of the 2X stocks of compound are added to the cells.

After 24 hours, the media is aspirated from the assay plates. Each well is rinsed with 1ml PBS, and the PBS is aspirated. 180μL

SDS-PAGE sample buffer (Novex) containing 5% 2-mercaptoethanol is added to each well. The plates are heated to 100°C for 5 minutes using a heat block containing an adapter for assay plates. The plates are placed on ice. After 10 minutes, 20µL of an RNAse/DNase mix is added per well. This mix is 1mg/ml DNaseI (Worthington Enzymes), 0.25mg/ml Rnase A (Worthington Enzymes), 0.5M Tris-HCl pH8.0 and 50mM $MgCl_2$. The plate is left on ice for 10 minutes. Samples are then either loaded on the gel, or stored at -70°C until use.

Each assay plate (usually 3 compounds, each in 4-point titrations, plus controls) requires one 15-well 14% Novex gel. 25µl of each sample is loaded onto the gel. The gel is run at 15mA for about 3.5 hours. It is important to run the gel far enough so that there will be adequate separation between 21kd (Rap1) and 29kd (Rab6).

The gels are then transferred to Novex pre-cut PVDF membranes for 1.5 hours at 30V (constant voltage). Immediately after transferring, the membranes are blocked overnight in 20ml Western blocking buffer (2% nonfat dry milk in Western wash buffer (PBS + 0.1% Tween-20). If blocked over the weekend, 0.02% sodium azide is added. The membranes are blocked at 4°C with slow rocking.

The blocking solution is discarded and 20ml fresh blocking solution containing the anti Rap1a antibody (Santa Cruz Biochemical SC1482) at 1:1000 (diluted in Western blocking buffer) and the anti Rab6 antibody (Santa Cruz Biochemical SC310) at 1:5000 (diluted in Western blocking buffer) are added. The membranes are incubated at room temperature for 1 hour with mild rocking. The blocking solution is then discarded and the membrane is washed 3 times with Western wash buffer for 15 minutes per wash. 20ml blocking solution containing

1:1000 (diluted in Western blocking buffer) each of two alkaline phosphatase conjugated antibodies (Alkaline phosphatase conjugated Anti-goat IgG and Alkaline phosphatase conjugated anti-rabbit IgG [Santa Cruz Biochemical]) is then added. The membrane is incubated for one hour and washed 3x as above.

About 2ml per gel of the Amersham ECF detection reagent is placed on an overhead transparency (ECF) and the PVDF membranes are placed face-down onto the detection reagent. This is incubated for one minute, then the membrane is placed onto a fresh transparency sheet.

The developed transparency sheet is scanned on a phosphorimager and the Rap1a Minimum Inhibitory Concentration is determined from the lowest concentration of compound that produces a detectable Rap1a Western signal. The Rap1a antibody used recognizes only unprenylated/unprocessed Rap1a, so that the precence of a detectable Rap1a Western signal is indicative of inhibition of Rap1a prenylation.

EXAMPLE 9

In vivo tumor growth inhibition assay (nude mouse)

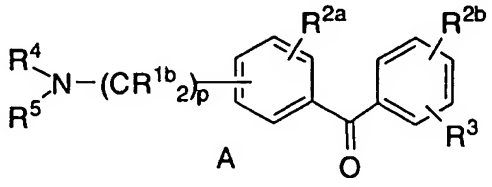
In vivo efficacy as an inhibitor of the growth of cancer cells may be confirmed by several protocols well known in the art. Examples of such *in vivo* efficacy studies are described by N. E. Kohl et al. (*Nature Medicine*, 1:792-797 (1995)) and N. E. Kohl et al. (*Proc. Nat. Acad. Sci. U.S.A.*, 91:9141-9145 (1994)).

Rodent fibroblasts transformed with oncogenically mutated human Ha-*ras* or Ki-*ras* (10^6 cells/animal in 1 ml of DMEM salts)

are injected subcutaneously into the left flank of 8-12 week old female nude mice (Harlan) on day 0. The mice in each oncogene group are randomly assigned to a vehicle, compound or combination treatment group. Animals are dosed subcutaneously starting on day 1 and daily for the duration of the experiment. Alternatively, the farnesyl-protein transferase inhibitor may be administered by a continuous infusion pump. Compound, compound combination or vehicle is delivered in a total volume of 0.1 ml. Tumors are excised and weighed when all of the vehicle-treated animals exhibited lesions of 0.5 - 1.0 cm in diameter, typically 11-15 days after the cells were injected. The average weight of the tumors in each treatment group for each cell line is calculated.

WHAT IS CLAIMED IS:

1. A compound which is a dual inhibitor of farnesyl-protein transferase and geranylgeranyl-protein transferees type I of the formula A:



wherein:

R^{1a} is selected from: hydrogen or C_1-C_6 alkyl;

R^{1b} is independently selected from:

a) hydrogen,

b) aryl, heterocycle, cycloalkyl, $R^{10}O$-, -$N(R^{10})_2$ or C_2-C_6 alkenyl,

c) C_1-C_6 alkyl unsubstituted or substituted by aryl, heterocycle, cycloalkyl, alkenyl, $R^{10}O$-, or -$N(R^{10})_2$;

R^{2a}, R^{2b} and R^3 are independently selected from:

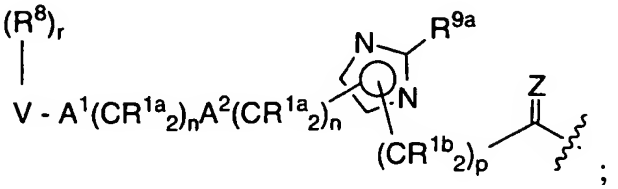
a) hydrogen,

b) C_1-C_6 alkyl unsubstituted or substituted by C_2-C_6 alkenyl, $R^{10}O$-, $R^{11}S(O)_m$-, $R^{10}C(O)NR^{10}$-, CN, N_3, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, $R^{10}OC(O)$-, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-,

c) unsubstituted or substituted aryl, unsubstituted or substituted heterocycle, unsubstituted or substituted cycloalkyl, alkenyl, $R^{10}O$-, $R^{11}S(O)_m$-, $R^{10}C(O)NR^{10}$-, CN, NO_2, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, $R^{10}OC(O)$-, N_3, -$N(R^{10})_2$, halogen or $R^{11}OC(O)NR^{10}$-, and

d) C_1-C_6 alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocyclic and C_3-C_{10} cycloalkyl;

R^4 is



;

R^5 is hydrogen;

R^8 is selected from:

a) hydrogen,

b) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 perfluoroalkyl, F, Cl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, CN, NO_2, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, $R^{10}OC(O)$-, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-, and

c) C_1-C_6 alkyl substituted by C_1-C_6 perfluoroalkyl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, $R^{10}OC(O)$-, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-;

R^{9a} is independently selected from C_1-C_6 alkyl and aryl;

R^{10} is independently selected from hydrogen, C_1-C_6 alkyl, C_1-C_6 perfluoroalkyl, 2,2,2-trifluoroethyl, benzyl and aryl;

R^{11} is independently selected from C_1-C_6 alkyl, benzyl and aryl;

A^1 and A^2 are independently selected from: a bond, -CH=CH-, -C≡C-, -C(O)-, -$C(O)NR^8$-, -$NR^8C(O)$-, O, -$N(R^8)$-, -$S(O)_2N(R^8)$-, -$N(R^8)S(O)_2$-, or $S(O)_m$;

V is selected from:

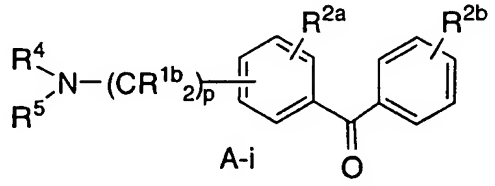
a) hydrogen,
b) heterocycle selected from pyrrolidinyl, imidazolyl, pyridinyl, thiazolyl, pyridonyl, 2-oxopiperidinyl, indolyl, quinolinyl, isoquinolinyl, and thienyl,
c) aryl,
d) C_1-C_{20} alkyl wherein from 0 to 4 carbon atoms are replaced with a heteroatom selected from O, S, and N, and
e) C_2-C_{20} alkenyl,

provided that V is not hydrogen if A^1 is $S(O)_m$ and V is not hydrogen if A^1 is a bond, n is 0 and A^2 is $S(O)_m$;

Z is H_2 or O;
m is 0, 1 or 2;
n is 0, 1, 2, 3 or 4;
p is independently 0, 1, 2, 3 or 4; and
r is 0 to 5, provided that r is 0 when V is hydrogen;

or a pharmaceutically acceptable salt thereof.

2. The compound according to Claim 1 of the formula A-i:



A-i

wherein:

R^{1b} is independently selected from:

a) hydrogen,
b) aryl, heterocycle, cycloalkyl, $R^{10}O$-, $-N(R^{10})_2$ or C_2-C_6 alkenyl,

c) C_1-C_6 alkyl unsubstituted or substituted by aryl, heterocycle, cycloalkyl, alkenyl, $R^{10}O$-, or -$N(R^{10})_2$;

R^{2a} and R^{2b} are independently selected from:

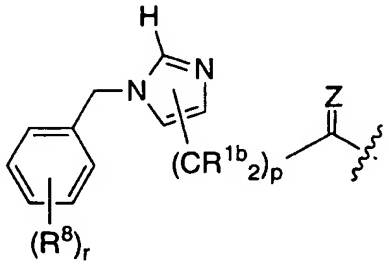
a) hydrogen,

b) C_1-C_6 alkyl unsubstituted or substituted by C_2-C_6 alkenyl, $R^{10}O$-, $R^{11}S(O)_m$-, $R^{10}C(O)NR^{10}$-, CN, N_3, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, $R^{10}OC(O)$-, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-,

c) unsubstituted or substituted aryl, unsubstituted or substituted heterocycle, unsubstituted or substituted cycloalkyl, alkenyl, $R^{10}O$-, $R^{11}S(O)_m$-, $R^{10}C(O)NR^{10}$-, CN, NO_2, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, $R^{10}OC(O)$-, N_3, -$N(R^{10})_2$, halogen or $R^{11}OC(O)NR^{10}$-, and

d) C_1-C_6 alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocyclic and C_3-C_{10} cycloalkyl;

R^4 is



;

R^5 is hydrogen;

R^8 is independently selected from:

a) hydrogen,

b) C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 perfluoroalkyl, F, Cl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, CN, NO_2,

$(R^{10})_2N$-C(NR^{10})-, $R^{10}C(O)$-, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-, and

c) C_1-C_6 alkyl substituted by C_1-C_6 perfluoroalkyl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, $(R^{10})_2N$-C(NR^{10})-, $R^{10}C(O)$-, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-;

R^{10} is independently selected from hydrogen, C_1-C_6 alkyl, substituted or unsubstituted C_1-C_6 aralkyl and substituted or unsubstituted aryl;

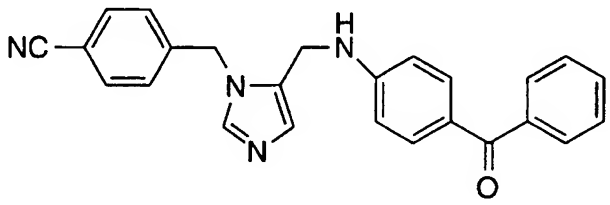
R^{11} is independently selected from C_1-C_6 alkyl, benzyl and aryl;

Z is H_2 or O;
m is 0, 1 or 2;
n is 0, 1, 2, 3 or 4;
p is independently 0, 1 or 2; and
r is 0 to 5;

or a pharmaceutically acceptable salt thereof.

3. A compound which inhibits farnesyl-protein transferase which is:

4-[((1-(4-cyanobenzyl)-5-imidazolyl)methyl)amino]benzophenone_



or a pharmaceutically acceptable salt or optical isomer thereof.

4. A pharmaceutical composition comprising a pharmaceutical carrier, and dispersed therein, a therapeutically effective amount of a compound of Claim 1.

5. A pharmaceutical composition comprising a pharmaceutical carrier, and dispersed therein, a therapeutically effective amount of a compound of Claim 2.

6. A pharmaceutical composition comprising a pharmaceutical carrier, and dispersed therein, a therapeutically effective amount of a compound of Claim 3.

7. A method for inhibiting farnesyl-protein transferase and geranylgeranyl-protein transferase type I which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 4.

8. A method for inhibiting farnesyl-protein transferase and geranylgeranyl-protein transferase type I which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 5.

9. A method for inhibiting farnesyl-protein transferase and geranylgeranyl-protein transferase type I which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 6.

10. A method for treating cancer which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 4.

11. A method according to Claim 10 wherein the cancer is characterized by a mutated K4B-Ras protein.

12. A method for treating cancer which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 5.

13. A method according to Claim 12 wherein the cancer is characterized by a mutated K4B-Ras protein.

14. A method for treating cancer which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 6.

15. A method according to Claim 14 wherein the cancer is characterized by a mutated K4B-Ras protein.

16. A method for treating neurofibromin benign proliferative disorder which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 4.

17. A method for treating blindness related to retinal vascularization which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 4.

18. A method for treating infections from hepatitis delta and related viruses which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 4.

19. A method for preventing restenosis which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 4.

20. A method for treating polycystic kidney disease which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 4.

21. A pharmaceutical composition made by combining the compound of Claim 1 and a pharmaceutically acceptable carrier.

22. A process for making a pharmaceutical composition comprising combining a compound of Claim 1 and a pharmaceutically acceptable carrier.

SEQUENCE LISTING

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Graham, Samuel L.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/17692

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07D 233/54, 233/60, 233/66, 233/90; A61K 31/415
US CL :548/336.1, 341.5; 514/399, 400
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/399, 400; 548/336.1, 340.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Please See Extra Sheet.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,758,573 A (MANLEY ET AL.) 19 July 1988, see entire document.	1-22
A	US 5,191,086 A (POSS) 02 March 1993, see entire document.	1-22

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 01 OCTOBER 1998	Date of mailing of the international search report 02 NOV 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer FLOYD D. HIGEL aco Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/17692

B. FIELDS SEARCHED
Documentation other than minimum documentation that are included in the fields searched:

Chemical Abstracts
Current Abstracts of Chemistry
Index Chemicus